RNAi of rpS3a suggests a link between this gene and arrested ovarian development during adult diapause in *Culex pipiens* (#1138)

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ABSTRACT

In mosquitoes that do not enter diapause, rpS3a is consistently expressed, but in mosquitoes programmed for diapause, expression of rpS3a is intermitted during early diapause, but is then consistently elevated in females 1 month and older. RNAi was performed to evaluate a possible function for rpS3a related to the arrested ovarian development during diapause. dsRNA injected into non-diapausing females suppressed ovarian development. Decreased expression of rpS3a following dsRNA injection was confirmed by Northern blot hybridization. Topical application of JHIII, an endocrine trigger for diapause termination in this species, yielded an almost complete recovery from the RNAi effect. We propose that rpS3a is involved in the shut down of ovarian development that characterizes the adult diapause of *Cx. pipiens*.

INTRODUCTION

• *Culex pipiens,* the Northern house mosquito, is known as one of main vectors of West Nile virus.

• Female mosquitoes in *Cx. pipiens* enter an adult diapause with the following characteristics:
- Programmed by the short day length of autumn (Sanburg and Larsen, 1973)
- Fat reserves as the energy source (Robich and Denlinger, 2005)
- Shutdown of JH (juvenile hormone) (Spielman, 1974)
- Arrested ovarian development during diapause (Christophers, 1911)

Nondiapause

Diapause

• Ribosomal protein S3a is a highly conserved protein that contributes to numerous physiological functions including:
- Inhibited ovarian development by suppressing rpS3a in *Drosophila melanogaster* (Reynaud et al., 1997)
- Up-regulation during oogenesis in *Anopheles gambiae* (Zurita et al., 1997)
- Down-regulation during diapause in *Cx. pipiens* (Robich et al., 2007)

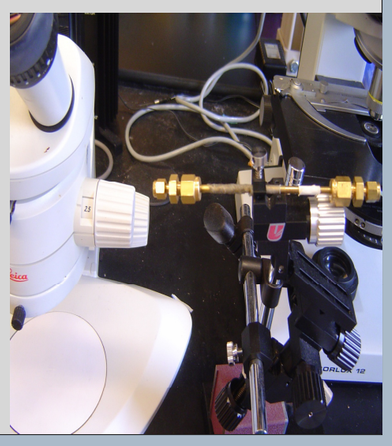
METHODS

• Insect rearing:
Nondiapause mosquitoes at 18°C with a 15Light:9Dark cycle
Diapause mosquitoes at 18°C with a 9Light :15Dark cycle

• Northern blot hybridization:
monitor rpS3a expression patterns

• RNAi procedure
(after Sim and Denlinger, 2008):
- dsRNA synthesis and injection:
inject dsRNA of rpS3a (400ng) to nondiapause females
- Measure the primary follicle size:
check the effect of RNAi

• Topical application of JHIII:
confirm the role of JHIII



Low expression of rpS3a in diapause females (7-10days)

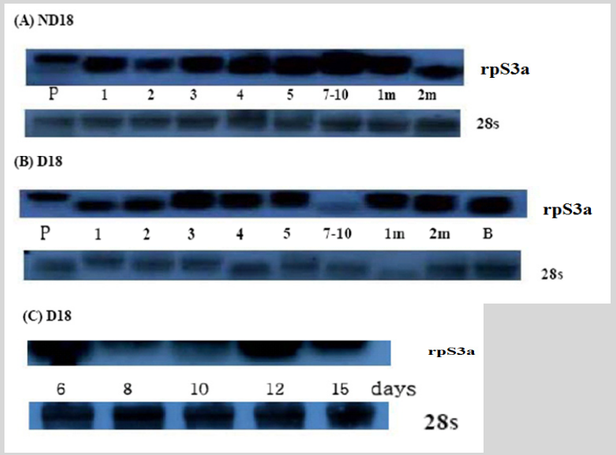


Figure 1. Northern blot hybridization of rpS3a. (A) RpS3a is continuously expressed in nondiapause mosquitoes. (B) In diapause mosquitoes, rpS3a is down-regulated in early (7-10 days) stage of diapause. (C) Further confirmation shows low expression of rpS3a on day 8 and 10. 28S ribosomal RNA is used as control.

Arrested ovarian development following dsRNA of rpS3a

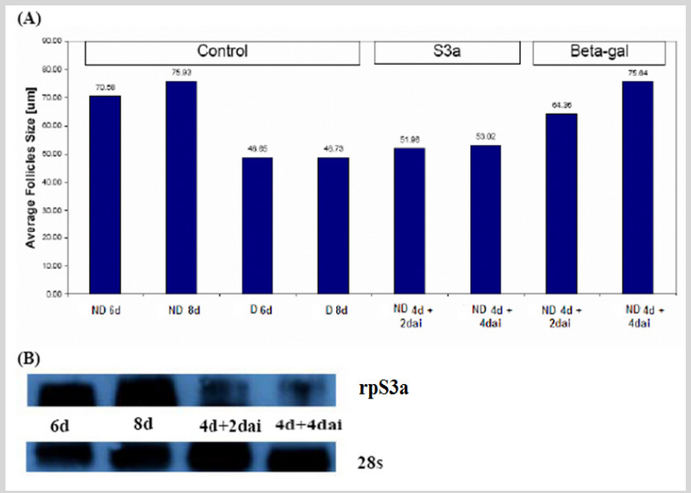


Figure 2. The effect of injection of dsRNA of rpS3a. (A) Primary follicle sizes after injection of dsRNA of rpS3a were measured (10 follicles measured per mosquito; total 10 mosquitoes for controls and 25 mosquitoes for others. ND = nondiapause mosquitoes, D = diapause mosquitoes, X days = X days after adult eclosion, X dai = X days after injection). Ovarian development was arrested when rpS3a was suppressed by dsRNA injection. dsRNA of βeta-galactose was injected and follicles measured for the internal control. (B) Northern blot hybridization results confirm the suppression of rpS3a in nondiapause mosquitoes. 28S ribosomal RNA is used as control.

RNAi against rpS3a suppresses follicle growth

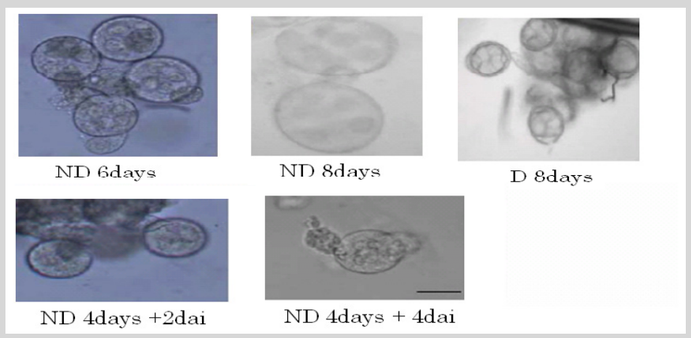


Figure 3. Primary follicle images (ND = nondiapause mosquitoes, D = diapause mosquitoes, X days = X days after adult eclosion, X dai = X days after injection)

RNAi effect degrades with time

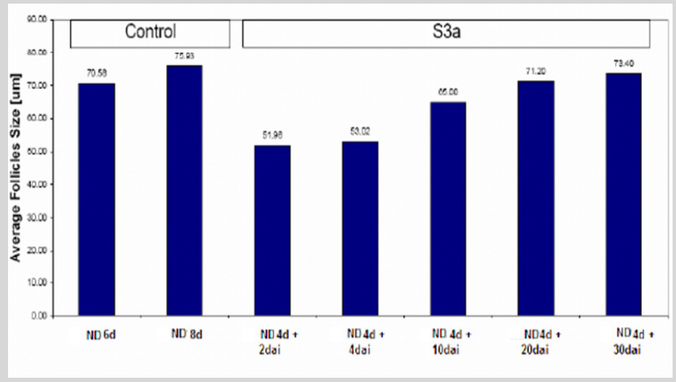


Figure 4. The effect of dsRNA of rpS3a injection with time. Primary follicle sizes were slightly increased with time after dsRNA of rpS3a injection due to the effect of degradation of dsRNA (10 follicles measured per mosquito; total 10 mosquitoes for controls and 25 mosquitoes for others. ND = nondiapause mosquitoes, D = diapause mosquitoes, X days = X days after adult eclosion, X dai = X days after injection).

JHIII rescues the arrested ovarian development

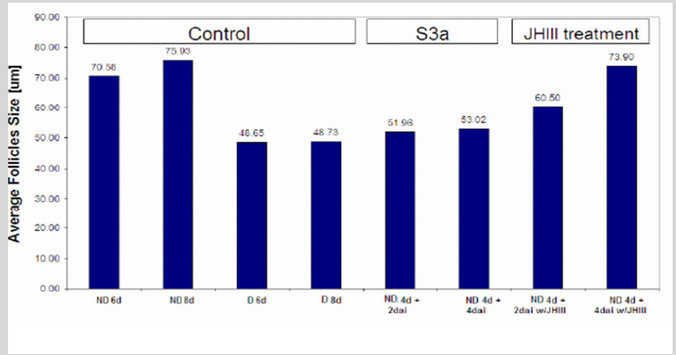


Figure 5. The role of JHIII as a trigger to terminate diapause. Primary follicles started to develop when JHIII were applied immediately after rpS3a injection (10 follicles measured per mosquito; total 10 mosquitoes for controls and 25 mosquitoes for others. ND = nondiapause mosquitoes, D = diapause mosquitoes, X days = X days after adult eclosion, X dai = X days after injection).

CONCLUSIONS

• RpS3a is down-regulated in early (8-10days after adult eclosion) diapause in female mosquitoes.

• RpS3a has a critical role in ovarian development of *Cx. pipiens*.

• JHIII can rescue the rpS3a suppressed mosquitoes.

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